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Prevalence incidence and clinical outcome of *Klebsiella* and *Acinetobacter* ventilator-associated pneumonia

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Abstract

Introduction: Antimicrobial resistance identification for each hospital has benefits in management of nosocomial infections.
Objectives: The aim of this study was to determine antimicrobial susceptibility of meropenem against *Acinetobacter* and *Klebsiella* strains in samples obtained from hospitalized patients with ventilator-associated pneumonia (VAP).
Patients and Methods: In this cross-sectional study, 100 patients with VAP were selected from the intensive care unit (ICU) of Amin hospital in Isfahan. Lung secretions were collected and tested for bacterial infections. In samples with the positive bacterial infection, E-test and agar diffusion test were used to determine and compare the susceptibility of *Acinetobacter* and *Klebsiella* strains to meropenem.
Results: The two susceptibility testing methods – E-test and agar diffusion test – showed similar results in 87 cases (87%) therefore, 84 cases were resistant and three cases were susceptible to meropenem. However, in 13 cases, the result of the agar diffusion test was resistant and the result of E-test was sensitive. According to the Kappa test, the agreement between the two tests was 0.87 and statistically significant (P <0.001).
Conclusion: Due to the high resistance of *Klebsiella* and *Acinetobacter* strains to meropenem, it should not be used as an experimental treatment in patients diagnosed with VAP caused by these strains. We recommend using meropenem for *Acinetobacter* and *Klebsiella* after susceptibility testing by E-test confirmed its efficacy.

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Introduction

One of the main causes of death and increased healthcare costs is infections in the intensive care unit (ICU). Due to the high prevalence of infections in this ward, antibiotics are used extensively which in many cases the desired outcome is not achieved. Therefore, selection of the effective treatment for patients in the ICU is very important (1).

Ventilator-associated pneumonia (VAP) that occurs 48 to 72 hours after endotracheal intubation is one of the most common Hospital-acquired infections. A previous study showed that about 10% of patients requiring mechanical ventilation develop VAP (2).

Studies have shown that mechanical ventilation increases from 7.6 days to 11.5 days, and hospital stay from 11.5 to 13.1 days in patients with VAP compared to patients without VAP. Longer hospital stay and ventilation incur an additional cost of approximately $40 000 per patient and has been associated with other complications such as respiratory failure, pleural effusion, and septic shock as well as mortality (3-7).

Key point

Each hospital has a certain amount of specific antibiotic resistance. Rapid treatment against resistance, and choosing the best antibiotics for the cure, are results of microbial typing.

Acinetobacter and *Klebsiella* strains are common gram-negative bacteria causing VAP. *Acinetobacter* is a gram-negative bacillus and its prevalence in Asia is about 19.2%. The most important species of this genus is *Acinetobacter baumannii*, which causes a variety of respiratory infections, urinary tract

infections, and ulcers, especially in the ICU (8-10)

Klebsiella pneumonia is also a gram-negative bacillus that is a common cause of infections in hospitalized patients and patients with weakened immune systems. The prevalence of this pathogen is 20.7% in ICUs of Asian countries (11)

Due to the high prevalence of VAP and its high morbidity and mortality as well as the high prevalence of these microorganisms in the ICU, the study of antibiotic resistance and VAP treatment is of great importance.

Objectives

According to safeguard the optimized environment in ICU, the nomination of antimicrobial susceptibility of meropenem against *Acinetobacter* and *Klebsiella* strains in samples obtained from lung secretions of patients provides an effective strategy for patient treatment.

Patients and Methods

Study design

This cross-sectional study was conducted in Amin hospital located in Isfahan during 2016-2017. The target population was patients admitted to the ICU. Inclusion criteria included VAP and the presence of *Acinetobacter* and *Klebsiella* in lung secretions. Patients with a colony count less than 10 000 CFU/mL were excluded from the study. We used bronchoscopy or tracheal suctioning for collecting lung secretions samples.

The sample size was estimated 100 using the sample size calculation formula for prevalence study considering the 95% confidence level, the prevalence of 0.5 for gram-negative bacteria infections, and an error rate of 0.1.

Lung secretions were cultured on blood agar and MacConkey agar (Merck). Bacteria from positive cultures were identified using microbiological and biochemical tests. Antibiograms of *Klebsiella* pneumonia and *A. baumannii* isolates were separately prepared by agar diffusion disk and E-Test using Mueller-Hinton agar.

Minimal inhibition concentration (MIC) was determined by the E-test (AB Biodisk, Sweden). The validity of the MIC values obtained for each microorganism using E-test kits was assessed by comparing them to the agar dilution, and broth microdilution references considering the National Committee for Clinical Laboratory Standards (NCCLS). If there was a consistency of more than 90% between E-test values compared to references, the E-test result was considered in agreement with the NCCLS reference method (essential agreement).

All experiments were conducted by a senior microbiologist using standard materials. Two reference strains with ATCC code were provided by Iranian reference laboratory. The results obtained from E-test were compared with the microbial growth ranges table provided by the reference laboratory and the samples were read at 24 hours at 37 degrees. The MIC was reported based on the point where the growth curve intersects the E-test strip. The results obtained from the E-test were compared with the numbers in the NCCLS standard table.

In the agar diffusion test, the kits were prepared from the MAST company (UK). The result of the susceptibility test was reported as resistant, intermediate, and sensitive in both E-test and agar diffusion test.

Statistical analysis

The results of the antibiogram test and patients' demographic information were analyzed in SPSS software version 25 using chi-square, *t* test, and diagnostic value tests (including sensitivity, specificity, false positive, false negative, and positive and negative predictive values). The result of E-test was considered the gold standard and was used for evaluating the validity of the agar diffusion test. $P < 0.05$ was considered as statistically significant.

Results

In this study, we analyzed 100 samples from lung secretions of patients in the ICU. Microbial culture of 55 and 45 samples grew the *Acinetobacter* and *Klebsiella*, respectively. The mean age of the patients was 60.2 ± 20.5 years (ranging from 13 to 91 years). The number of male and female patients was 64 and 36, respectively. Head trauma was the most common cause of ICU admission, with a frequency of 38 cases. During the study, 52 patients died, and cerebral hemorrhage was the leading cause of death in 18 patients (34.6%).

Table 1 shows the distribution of demographic and clinical characteristics of all the patients and by type of VAP-causing bacteria. According to the table, the mean age of the two groups with *Acinetobacter* and *Klebsiella* was not significantly different. The prevalence of both types of bacteria was higher in males than females; however, the difference was not significant. In both types of bacteria, trauma was the most common cause of hospitalization with a prevalence of 27.3% and 53.3% in patients infected with *Acinetobacter* and *Klebsiella*, respectively. The type of bacteria for different causes of hospitalization was not significantly different.

The incidence of death in patients with *Acinetobacter* was 50.9% and in patients with *Klebsiella* was 53.3%. The difference between different types of bacteria was not significant. The leading cause of death in both types of bacterial infections was cerebral hemorrhage with a frequency of 25% in *Acinetobacter* infections and 45.8% in *Klebsiella* infections. There was no significant difference between the two types of bacterial infections.

The results of the agar diffusion test showed that 97 out of 100 samples were resistant to meropenem, and three were sensitive. According to the test, all 55 samples infected with *Acinetobacter* were resistant to meropenem; however, 42 (93.3%) and three (6.7%) out of 45 samples infected with *Klebsiella* (93.3%) were resistant and sensitive, respectively. The resistance to meropenem was not significantly different between the two bacteria (*P*

Table 1. Distribution of demographic and clinical characteristics of all the patients and by the type of VAP-causing bacteria

Variable		All the patients	Type of bacteria		*P* value
			Acinetobacter	*Klebsiella*	
Mean of age (y)		60.2± 20.5	60.1±22.4	60.3±18.6	0.98
Gender	Male	64 (64%)	35 (63.6)	29 (64.4)	0.93
	Female	36 (36%)	20 (36.4)	16 (35.6)	
Cause of ICU admission	Heart disease	9 (9%)	5 (9.1)	4 (8.9)	0.42
	Stroke	9 (9%)	6 (10.6)	3 (6.7)	
	Trauma	38 (38%)	15 (27.3)	23 (51.1)	
	Cerebral hemorrhage	6 (6%)	4 (7.3)	2 (4.4)	
	Infection	10 (10%)	5 (9.1)	5 (11.1)	
	Chronic obstructive pulmonary disease	4 (4%)	3 (5.5%)	2.2) 1)	
	Pneumonia	5 (5%)	4 (7.3)	2.2) 1)	
	Respiratory diseases	8 (8%)	5 (9.1)	3 (6.7)	
	Others	11 (11%)	8 (14.5)	3 (6.7)	
Patient's outcome	Discharge	48 (48%)	27 (49.1)	21 (46.7)	0.81
	Death	52 (52%)	28 (50.9)	24 (53.3)	
Cause of death	Septicemia	7 (13.5)	4 (14.3)	3 (12.5)	0.32
	Heart	7 (13.5)	4 (14.3)	3 (12.5)	
	Respiratory	6 (11.5)	2 (7.1)	4 (16.7)	
	Pneumonia	5 (9.6)	3 (10.7)	2 (8.3)	
	Cerebral hemorrhage	18 (34.6)	7(25)	11 (45.8)	
	Stroke	3 (5.8)	3 (10.7)	0 (0)	
	Digestive and liver	3 (5.8)	3 (10.7)	0 (0)	
	Others	3 (5.8)	2 (7.1)	1 (4.2)	

= 0.09). According to the results of the E-test, 84 out of 100 samples were resistant to meropenem, and 16 were sensitive. According to the test, 52 samples (94.5%) infected with *Acinetobacter* and 32 samples (71.1%) infected with *Klebsiella* were resistant to meropenem. The resistance of two types of bacteria was significantly different ($P = 0.001$; Table 2).

According to our study, 87 out of 100 samples (87%) had similar results using agar diffusion test and E-test; therefore, 84 samples were resistant, and three samples were sensitive to meropenem. We observed discordant results in 13 samples that were resistant using agar diffusion test and sensitive using E-test. According to the Kappa test, the agreement between the two tests was 0.87 and was statistically significant ($P <0.001$).

Examination of the agreement between the two tests based on the type of bacteria showed that the results of the two tests were similar in 55 samples infected with *Acinetobacter*. However, in 45 samples infected with *Klebsiella*, the results of the two tests were similar in 35 samples (77.8%) and discordant in 10 samples (22.2%) so that agar diffusion test was resistant and E-test was sensitive (Table 3).

According to our results, the susceptibility test of *Acinetobacter* and *Klebsiella* using agar diffusion test has a sensitivity of 100% (resistance to meropenem) and a specificity of 18.8% (sensitivity to meropenem). On the other hand, the test has 81.3% false negatives and zero false positive error. The positive predictive value of disk diffusion test is 86.6% and its negative predictive value is 100%. The accuracy of the test was 87%.

The susceptibility test of *Acinetobacter* and *Klebsiella* using agar diffusion test has a sensitivity of 100%; however, its specificity was zero for *Acinetobacter* and 23.1% for *Klebsiella*. The positive predictive value of the test was 94.5% for *Acinetobacter* and 76.2% for *Klebsiella*. The negative predictive value for *Acinetobacter* and *Klebsiella* was zero and 100%, respectively (Table 4).

Table 2. Frequency distribution of *Acinetobacter* and *Klebsiella* resistance to meropenem using agar diffusion method and E-test

Type of test	Outcome	Type of bacteria	
		Acinetobacter	*Klebsiella*
Disk-diffusion Agar method	Resistant	55(100)	42 (93.3)
	Sensitive	0(0)	3(6.7)
E-test	Resistant	52 (94.5)	32 (71.1)
	Sensitive	3 (5.5)	13(28.9)

Table 3. Agreement between diffusion and E-test in determining antibiotic resistance to meropenem

Type of bacteria	E-test	Disk diffusion		Agreement	*P* value
		Resistant	Sensitivity		
All samples	Resistant	84 (84%)	13 (13%)	0.87	<0.001
	Sensitive	0 (0%)	3 (3%)		
Acinetobacter	Resistant	55 (94.5%)	0 (0%)	>0.99	>0.99
	Sensitive	0 (0%)	3 (5.5%)		
Klebsiella	Resistant	32 (71.1%)	10 (22.2%)	8.77	<0.001
	Sensitive	0 (0%)	3 (6.7%)		

Discussion

Preparing an antibiogram is very important for determining the antimicrobial susceptibility of VAP-causing microbial strains in patients admitted to the ICU. Failure to detect bacterial sensitivity to the prescribed antibiotic can lead to uncontrolled infection, and ultimately death or a prolonged ICU stay and secondary complications (12).

In this study, we assessed one hundred samples of VAP-causing *Acinetobacter* and *Klebsiella* with agar diffusion test and E-test. Our results showed that a significant percentage of the samples (13%) that were resistant to meropenem using agar diffusion test was sensitive to meropenem by the E-test, which has a higher sensitivity and reliability. E-test leads to a more accurate diagnosis of antibiotic susceptibility and prevents the administration of stronger expensive antibiotics.

In other words, although E-test is more time-consuming and expensive than the agar diffusion test, its accuracy in detecting antibiotic resistance prevents unnecessary use of stronger antibiotics than meropenem. Using this test ultimately reduces hospital costs, mortality and morbidity in ICU, length of stay in the ICU, the incidence of antibiotic-induced side effects, and resistant bacteria spread in this unit.

The importance of E-test in determining the antibiotic resistance of different microbial strains is widely accepted. Several studies have reported E-test as a gold standard for measuring antibiotic resistance of gram-positive and gram-negative bacteria (13). The aim of our study was not determining the diagnostic value of disk diffusion compared to E-test. We conducted this study to verify the use of the agar diffusion test for detection of antibiotic resistance of two common gram-negative VAP-causing strains to meropenem, routinely used in VAP treatment, is an error-prone procedure which incurs cost and leads to loss of golden time for patients' treatment because of its inaccuracy in detecting antibiotic resistance of *Klebsiella* and *Acinetobacter*.

Table 4. Criteria for diagnostic value of disk diffusion test in determining resistance to meropenem

Type of test	All samples	*Acinetobacter*	*Klebsiella*
Sensitivity	100	100	100
Specificity	18.8	0	23.1
False positive	81.3	100	76.9
False negative	0	0	0
Positive predictive value	86.6	94.5	76.2
Negative predictive value	100	0	100
Test accuracy	87	97.5	77.8

Evaluation of agar diffusion diagnostic value compared to the E-test showed that this test has an overall sensitivity of 100% that means it detects real antibiotic resistance; however, its sensitivity, which indicates a sensitivity to meropenem, is about 18.8%. On the other hand, in clinical decision-making, the results of our study showed that the positive predictive value of disk diffusion is 86.6%. In other words, if a sample was resistant to meropenem using disk diffusion, the probability that the strain is resistant to meropenem is about 86.6%. This predictive value could make clinical decision-making for meropenem prescription or stronger antibiotics challenging. The diagnostic value of agar diffusion test and E-test in antibiotic susceptibility testing of other strains have also been studied (14). Direkel et al compared the sensitivity of agar diffusion test and E-test in determining the antibiotic susceptibility of Pseudomonas aeruginosa to penicillin/tazobactam. In this study, the sensitivity to penicillin/tazobactam was 64% and 36% using the E-test method and agar diffusion test, respectively (15). The inaccuracy of the antibiotic susceptibility test using the agar diffusion test was significantly higher. Our results were similar to this study.

Identifying drug-resistant gram-negative bacteria in patients with VAP in a short time is critical for implementing timely infection control measurements. Tests used must be highly effective, especially in detecting carbapenem hydrolysis by different classes of enzymes that can affect efficiency of different tests to identify multiple isolates producing carbapenemase (16). For example, two studies reported that the MICs of carbapenems was high while the susceptibility range was low in *A. baumannii* and Enterobacteriaceae (17,18). Therefore, using precise methods that could also be expensive to determine the antibiotic susceptibility of gram-negative strains in VAP patients is worthwhile in hospitals. The use of robust and reliable methods such as E-test is preferable to conventional

diagnostic methods such as agar diffusion for controlling the spread of these strains as well as in screening programs. This study aimed to determine whether the high antibiotic resistance to the meropenem reported in the laboratory was due to an inherent error and less sensitivity of the agar disk diffusion method compared to the E-test method. The findings of the present study showed that there is no laboratory error in reporting the results of resistance to this antibiotic. However, further studies to determine the effectiveness of this antibiotic in treating patients with VAP and determining whether or not these results match laboratory results is recommended.

Conclusion

According to our results, *Klebsiella* and *Acinetobacter* strains are resistant to meropenem. Therefore, this antibiotic should not be administered as an experimental treatment for patients with VAP, who are infected with *Klebsiella* and *Acinetobacter* strains, hospitalized in Amin hospital. Meropenem should be prescribed after the antibiogram test confirmed *Klebsiella* and *Acinetobacter* strains are sensitive.

Limitations of the study

Sample collection from patients' lungs is difficult. The special antibiotic survey is another limitation. Therefore, further studies on this subject are necessary.

Authors' contribution

Conceptualization: HM.
Methodology: HM, AH, MRV.
Validation: MRV, AH.
Formal analysis: MS, SY.
Investigation: SY.
Resources: SF, MS.
Data curation: SF.
Writing—original draft preparation: MS.
Writing—review and editing: SF.
Visualization: HM, SF.
Supervision: HM.
Project administration: HM, MS.
Funding acquisition: HM.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical issues

This research considered and observed all the principles of the Helsinki Declaration and was approved by the ethics committee of Isfahan university of medical sciences (#IR.MUI.REC.1395.3.764). Accordingly, written informed consent was taken from all participants or their parents before any intervention. This study was extracted from M.D., thesis of Mohammad Soleimani at this university (Thesis #395794). Moreover, ethical issues (including plagiarism, data fabrication, double publication) were completely observed by the authors.

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